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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
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| EXAMINER | |
|---|---|
| SWITZER, JULIET CAROLINE | |
| **ART UNIT** | **PAPER NUMBER** |
| 1634 | |

DATE MAILED: 08/17/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

| Advisory Action | Application No. | Applicant(s) | |
|---|---|---|---|
| | 09/545,162 | LAPIDUS ET AL. | |
| | Examiner | Art Unit | |
| | Juliet C. Switzer | 1634 | |

*--The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

THE REPLY FILED 02 August 2004 FAILS TO PLACE THIS APPLICATION IN CONDITION FOR ALLOWANCE. Therefore, further action by the applicant is required to avoid abandonment of this application. A proper reply to a final rejection under 37 CFR 1.113 may only be either: (1) a timely filed amendment which places the application in condition for allowance; (2) a timely filed Notice of Appeal (with appeal fee); or (3) a timely filed Request for Continued Examination (RCE) in compliance with 37 CFR 1.114.

PERIOD FOR REPLY [check either a) or b)]

a) ☒ The period for reply expires 4 months from the mailing date of the final rejection.

b) ☐ The period for reply expires on: (1) the mailing date of this Advisory Action, or (2) the date set forth in the final rejection, whichever is later. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of the final rejection.
ONLY CHECK THIS BOX WHEN THE FIRST REPLY WAS FILED WITHIN TWO MONTHS OF THE FINAL REJECTION. See MPEP 706.07(f).

Extensions of time may be obtained under 37 CFR 1.136(a). The date on which the petition under 37 CFR 1.136(a) and the appropriate extension fee have been filed is the date for purposes of determining the period of extension and the corresponding amount of the fee. The appropriate extension fee under 37 CFR 1.17(a) is calculated from: (1) the expiration date of the shortened statutory period for reply originally set in the final Office action; or (2) as set forth in (b) above, if checked. Any reply received by the Office later than three months after the mailing date of the final rejection, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

1. ☐ A Notice of Appeal was filed on _____. Appellant's Brief must be filed within the period set forth in 37 CFR 1.192(a), or any extension thereof (37 CFR 1.191(d)), to avoid dismissal of the appeal.

2. ☒ The proposed amendment(s) will not be entered because:

   (a) ☒ they raise new issues that would require further consideration and/or search (see NOTE below);

   (b) ☐ they raise the issue of new matter (see Note below);

   (c) ☒ they are not deemed to place the application in better form for appeal by materially reducing or simplifying the issues for appeal; and/or

   (d) ☐ they present additional claims without canceling a corresponding number of finally rejected claims.

   NOTE: *See Continuation Sheet*.

3. ☐ Applicant's reply has overcome the following rejection(s): _____.

4. ☐ Newly proposed or amended claim(s) _____ would be allowable if submitted in a separate, timely filed amendment canceling the non-allowable claim(s).

5. ☒ The a)☐ affidavit, b)☐ exhibit, or c)☒ request for reconsideration has been considered but does NOT place the application in condition for allowance because: *See Continuation Sheet*.

6. ☐ The affidavit or exhibit will NOT be considered because it is not directed SOLELY to issues which were newly raised by the Examiner in the final rejection.

7. ☐ For purposes of Appeal, the proposed amendment(s) a)☐ will not be entered or b)☐ will be entered and an explanation of how the new or amended claims would be rejected is provided below or appended.

   The status of the claim(s) is (or will be) as follows:

   Claim(s) allowed: _____.

   Claim(s) objected to: _____.

   Claim(s) rejected: _____.

   Claim(s) withdrawn from consideration: _____.

8. ☐ The drawing correction filed on _____ is a)☐ approved or b)☐ disapproved by the Examiner.

9. ☐ Note the attached Information Disclosure Statement(s)( PTO-1449) Paper No(s). _____.

10. ☐ Other: _____

Juliet C. Switzer

JULIET C. SWITZER
PATENT EXAMINER

Continuation of 2. NOTE: The preamble as recited introduces a new consideration under 112 2nd paragraph because it is not clear if "a colorectal" modifies cancer only or both cancer and precancer. Furthermore, claim 8 is confusing and raises a potential issue of new matter over the reciation "said long nucleic acid is a degradation product of DNA that is presen tin both normal and cancerous or precancerous cells"

Continuation of 5. does NOT place the application in condition for allowance because: The remarks are directed towards the amendments that were not entered. It is noted, however,

# SPECIFIC INHIBITION OF THE POLYMERASE CHAIN REACTION USING A NON-EXTENDABLE OLIGONUCLEOTIDE BLOCKER

## REFERENCE TO GRANTS

The work associated with this application was supported in part by grants AR33278 and AR39939 from the National Institute of Health.

## FIELD OF THE INVENTION

This invention relates to a method for selectively inhibiting the amplification of DNA sequences using the polymerase chain reaction (PCR). More particularly, this invention relates to a novel method for inhibiting amplification of a nucleic acid template by binding a non-extendable oligonucleotide blocker to the inter-primer region of a nucleic acid template.

This technique has both clinical and research applications.

## BACKGROUND OF THE INVENTION

DNA analysis is currently used in many research, diagnostic and forensic procedures. Some research strategies currently used include the polymerase chain reaction with broad range primers for amplifying bacterial targets, representational difference analysis and genotype determination.

For many reasons, it is important to be able to identify the genus, species or other taxonomic classification to which a bacterium belongs, or to be able to rapidly and accurately identify a tissue genotype. For example, such classification is important in order to distinguish different disease causing pathogens, such as in septic arthritis. It is imperative that taxonomic identification be expedient for pathogenic organisms, such as viruses, bacteria, protozoa, and multicellular parasites. This will assist in diagnosis and treatment of human and animal diseases, as well as studies in epidemiology and ecology.

Traditionally the identification of bacterial species, viruses, protozoa, and multicellular parasites has been achieved by studying the morphology, growth requirements, fermentation patterns, and antibiotic sensitivity of the pathogenic organisms as well as by immunologic methods. These methods are time consuming and require highly trained personnel. Moreover, the presence of some organisms cannot be recognized by these methods. More recently, methods have been developed for identification of pathogenic organisms by detection of RNA and DNA sequences using the polymerase chain reaction (PCR).

The recent development of the PCR has provided an important tool for the detection of nucleic acid sequences at low concentrations. The method allows amplification of a selected target region of DNA by providing two DNA primers. Each primer is complementary to a base sequence on one of the two complementary DNA strands, located at the ends of the target region. Each primer can hybridize to its complementary site after the DNA strands are separated. The 5' ends of the single-stranded primers are then extended using DNA polymerase to form double-stranded molecules that reach at least as far as the region complementary to the other primer. The double-stranded molecules are then separated by heat denaturation again, and the process is repeated.

Repetition of this process a number of times results in the exponential generation of multiple copies of DNA that correspond in sequence to the target region. Accordingly, a small number of target sequences can be exponentially amplified and the resulting large number of molecules then reliably detected.



Some recent applications of the PCR have employed primers that are complementary to more than one DNA target and that therefore can amplify multiple such sequences differing mainly in the sequences between those complementary to the primers, called here the inter-primer region. One such application takes advantage of the fact that some DNA sequences in the bacterial genes that code for ribosomal RNA's are phylogenetically conserved among different species. The degree of conservation varies with the location within these genes. Thus some such sequences are common to groups of phylogenetically related species. The sizes of those phylogenetic groups can vary greatly and can include, for example, only very closely related species or, at the other extreme, can include all species of bacteria. One can therefore use one primer pair to amplify DNA from any member of such a related group of bacterial species whose presence might be suspected in a clinical specimen. The identity of the species actually amplified can then be narrowed down further.

The latter can be accomplished by several methods including use of additional PCR's with primers that target smaller subgroups of bacteria, Southern blotting and analysis of restriction fragment sites. Many of these methods are technically difficult, expensive and time consuming. An alternative method of narrowing down the identity of such an amplified bacterial DNA would be by use of the present invention to specifically block amplification of selected members of the group of DNA's that can be amplified by that pair of primers.

Another possible application of the present invention is in use of the PCR for genotyping where two allotypes are both amplified by the same primers and differ by an insertion that is too short to allow their amplicons to be easily distinguished by size alone. Amplification of the allotype possessing the insert could be blocked specifically, allowing them to be distinguished. Still another possible use for the present invention is in representational difference analysis (RDA) where amplification of selected sequences could be blocked, for example, at the stage of preparing a genomic representation.

Such sequence-dependent blocking can be achieved by using a method developed by Henrik Orum et al. which utilizes peptide nucleic acids (PNA) which bind to specific regions on a DNA template to form a PNA/DNA complex. The PNA/DNA complex formed is more stable than a DNA/DNA complex and PNA cannot act as a primer. A specific PNA complementary to an inter-primer region on a nucleic acid sequence is synthesized and allowed to hybridize to the specific DNA inter-primer region during PCR amplification. Once the PNA/DNA complex is formed, amplification is blocked since the PNA is unable to be extended or removed.

This process is limited in that PNA are not easily synthesized and therefore are not readily available. Moreover, the per base cost is much more expensive than a corresponding DNA oligonucleotide blocker. Therefore this method is not economically efficient or widely available.

The present invention describes an alternative method by which an easily synthesized oligonucleotide blocker that binds specifically to a sequence in the inter-primer region can block the amplification of that target by preventing extension of one of the primers. To accomplish this, the blocking oligonucleotide is made non-extendable and therefore cannot itself act as primer which might result in